ISSN 110-2047. ALEX. J. VET.SCIENCE. 2001 , VOL 17 No.1 :97-117

PROPHYLACTIC EFFECT OF GARLIC AGAINST AFLATOXICOSIS IN JAPANESE QUAIL

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ABSTRACT Garlic improved significantly ($p<0.001$) the body weight and the feed conversion in quails when used alone or added to aflatoxicated feed at 0.25 or 0.50 ppm levels. Both levels of garlic decreased the mortality due to aflatoxins. Fresh minced garlic increased significantly ($p<0.001$) the hemoglobin and hematocrite values in non toxicated groups and preserved these values in aflatoxicated groups. Addition of garlic at both levels (2 & 4%) not only diminshed significantly ($p<0.001$) the adverse effects on ALT and AST during aflatoxicosis but also improved the liver functions in non toxicated birds, while total serum proteins, globulins and albumins were increased in garlic treated birds. A sharp decrease of these parameters due to aflatoxins was reported. Although garlic at 2% and 4% led to higher HI antibody response to NDV control non toxicated groups than other groups, there was no significant reduction in the immune response in the intoxicated groups. Liver was the most sensitive organ to the adverse effects of aflatoxin where the decrease in the organ weights was more pronounced than the decrease of the body weight while change in bursa weights were parallel to the changes in the body weight. Moreover, garlic at both levels had improved the histopathologic picture in the liver due to aflatoxicosis.

Introduction

Feed is a potent source of aflatoxin producing Aspergilli which constitute a big threat to the poultry industry (Ellakany, 1991).In Japanese quail aflatoxin causes poor growth, decrease of feed utilisation, decrease of egg production and delayed sexual maturity in both sexes and decrease of hatchability, egg quality and egg weight (Sawhney et al., 1973, Doerr and Ottinger 1980).

Aflatoxin in chickens causes immunosuppresion so increases the severity of cecal coccidiosis, Marek's disease and salmonellosis (Edds et al., 1973). Furthermore, aflatoxins causes anaemia as it decreases packed cell volume (PCV), RBCs counts and hemoglobin concentration (Hb) (Lanza et al., 1981, El-Shaarawi 1989). Aflatoxin decreases total serum protein, lipoprotein, cholesterol, triglycerides, lactate dehydrogenase, alanine aminotransferase (ALT), sorbitol

dehydrogenase and glutamic dehydrogenase (Tung et al., 1972, Doerr et al., 1983; Huff et al., 1986,.Dafalla et al., 1987, Abd El-Hamid et al., 1992 and Fernandez et al., 1994). Higher levels of gamaglutamil transferase (GGT), lactic dehydrogenase (LDH) and alanine asparatate transaminase (AST) due to aflatoxin were recorded by Brugere-Picoux et al., 1987, Balachandran and Ramarkrishian 1988 and Abd El-Hamid et al., 1992.

Aflatoxins also causes atrophy of the liver due to failure to mature (Huff et al., 1986). In addition, aflatoxins was thought to be the cause of fatty liver syndrome that is characterized by friable, large and yellow liver (Hamilton and Garlich 1971).

Garlic at 2 and 4% in feed increased excretion of neutral fat and acidic steroids, so decreased level of lipids, lipoproteins, cholesterol and liver weight in rats experimentally fed fat by about 30% (Myung Chi et al., 1982). Garlic increased the clotting time (Jain, 1977)

Garlic adminstration decreased the blood glucose levels and alkaline phosphatase, increased the antibody titers to sheep RBCs, while no effect was observed on the AST & ALT levels (El-Habbak et al., 1989).

This work was conducted to evaluate the effect of adding fresh minced garlic cloves to the aflatoxicated feed on liver function, live body weight, feed conversion, some blood and serum parameters, immune response to the ND vaccination and the histopathological picture in Japanese quail.

Material and Method

Experimental birds

A total number of 675 Japanese quail, 2 weeks old, were brought to Dept. Poultry Production, Fac. Agriculture, Kafr El-Sheikh, Tanta Univ. where they were wing tagged, weighed and placed on wooden wire floored boxes of 70x70x50 cm dimensions throughout the experiment. Feed and water were available ad libitum. The ration was formulated according to the NRC 1994 standards (protein 24%, Energy 2900 kcl/kg, Ca 0.8%, P 0.4%). The ration was tested and found to be free from aflatoxin.

Production of aflatoxin and mixing of feed

Aflatoxin was produced by growing *A.flavus* standard toxigenic strain, on sterile polished rice by the method of Shotwell et al. (1966) and as modified by West et al. (1973). Rice was cleaned, washed and autoclaved at 121°C for 15 minutes, dispended in a 500 ml Earlynmyer flasks and moistened by distelled water (10 ml/flask). Each flask was infected by a fresh spore saline suspension of *A.flavus*, then sealed by a cotton plug and incubated at 18°C for 2 days and then at 20°C for another 2 days followed by 3 days at 26°C. The flasks were shaken vigorously every day to prevent the clumping of the

rice and to insure a homogenous toxin production. Finally, the flasks were sterilized by autoclaving to kill the fungas while the toxins were restored. Then, the rice was dried and ground in an electric blender until being powder and then assayed using affinity column chromatography.

Detection of aflatoxins

Aflatoxins were detected quantitatively by using affinity column chromatography (Aflatest 10, Naremco, Springfield, U.S.A.) and flurometer (Sequcia Tuner Model 450 with 360 nm excitation filter and 450 nm emission filter). The procedure was as follows:

1-Weigh 50 g of examined sample into a blender jar. Add 100 ml of 80% methanol (20% water), blend at high speed for 1 minute. Pour 10 ml filtered extract into a clean vessle and dilute the extract with 40 ml of distelled water and mix well.

2- Filter the diluted extract through glass microfibre filter (Whatmann filter No. 2).

3-Transfer 10 ml of the filtrate into 10 ml syringe and push the amount through (Aflatest 10) column using the syringe pump.

4-Wash the column twice with 10 dist. water using the syringe pump.

5-Using the syringe pump quickly push one ml of HPLC methanol through the column and receive the elute into the glass cuvette of the flurometer.

6-Add 1 ml aflatest developer solution (0.002% bromine) to the cuvette and mix the contents by the vortex mixer for 10-15 seconds.

7-Place the cuvette into the flurometer which has been calibrated with the aflatest standard. The digital readout of the flurometer presents ppb of aflatoxins.

The aflatoxicated rice was added to the final feed to give a concentration of 0.25 and 0.5 ppm in the feed. Rice addition in the final feed did not exceed 1%.

Preparation of garlic

Garlic husks were removed, the cloves were separated and then chopped in an electric mixer where it was minced to a fine homogenate. This homogenate was prepared freshly every week and mixed with feed at the appropriate proportion.

Blood testing for NDV using HI test:

Serum samples were collected at 14 day old (before the start of the treatment), 28, 35 and 49 days of age for HI titers against NDVaccording to the method described by Anon (1980).

Biochemical traits

At 28 and 49 days of age, blood samples were collected and serum was separated for evaluation of liver function and for haematology (haemoglobin and haematocrite).
Alanino aminotransferase (ALT) and alanine asparatate aminotransferase

(AST) were evaluated by colourimetric methods using commercial kits supplied from Biomerieux (Poains, France).
Total serum protein was determined according to Weichselbaum (1946) and albumin after Bartholomev and Delancy (1966). Cholesterol was also estimated according to Folch et al. (1957).

Pathological studies:

Following complete necropsy, fresh specimens from the liver of killed birds were collected then rapidly fixed in 10% neutral buffered formosaline, processed through the conventional paraffin embedding technique. Paraffin sections of about 5 microns thickness were stained with haematoxylene and eosin (H&E) according to the method described by Culling (1983).

Experimental Design

A total number of 675 Japanese quails (2 weeks old) were divided randomly into 9 treatment groups with three replicates of 25 birds in each replicate. Each group was randomly alloted to one of the following treatments:

Experimental group	Level of garlic (1)	Level of Aflatoxin (2)
1	-	-
2	2%	-
3	4%	-
4	2%	0.25 ppm
5	4%	0.50 ppm
6	4%	0.25 ppm
7	2%	0.50 ppm
8	-	0.25 ppm
9	-	0.50 ppm

(1) Garlic was freshly minced weekly and added as indicated.
(2) The aflatoxicated feed and garlic was added for 5 weeks (14-49 days old)

All the different treatments were vaccinated according to the following program:
At 14 days of age, they received Hitchner B1 and LaSota at 21, 28 and 40 days of age.
The vaccines were adminstered using eye drop method.

Weekly feed consumption and body weight as well as daily mortality were recorded through the experimental period. At 28, 35 and 49 days of age, 5 birds from each replicate were weighed and bled either for assessment of the hematology (heparinized blood), enzymatic examination (serum), or for HI test for NDV. Then killed and the liver, spleen, intestine and bursa of Fabricius were removed, weighed and the relative organ body weight ratio were calculated. At 49 days of age, the remaining birds were bled, necropsied and specimens from liver

Were collected for the histopathological examination.

Statistical analysis

The data for all parameters were statistically analysed after SAS (1988) -

Statistical model

$yij = \mu + Ti + ej$

yij =Observation of the parameter

μ =Overall mean

Ti =Effect of treatment (i=1-9)

ej =Random error treatment

Results

Effect of treatments on body weight and feed conversion

As shown in table (1), addition of aflatoxin (AF) at the level of 0.25 or 0.5 ppm significantly depressed the body weight (grp 8,9) when compared with the non aflatoxicated, non garlic treated group (grp 1). However, not only addition of garlic at 4% and 2% in the feed gave the highest body weight by the end of the experiment (at 49 days of age) (grp 3,2), but also improved significantly ($p<0.001$) the body weight in both intoxicated groups (grp 4,5,6,7).

Regarding the feed conversion (table 2), it was clear that feeding garlic at 4% level produced the lowest feed conversion ratio (grp 3). On contrary, AF at 0.50 ppm produced the highest feed conversion ratio (grp 9). Addition of garlic at 2% or 4% to the aflatoxicated feed which contained either 0.25 ppm or 0.50 ppm resulted in significant ($p<0.001$) improvement in the feed conversion.

Mortality rate

From table 3, it was evident that there was a positive correlation between the mortality and the dose of the aflatoxin. It is also clear that both levels of inclusion rate of garlic decreased the mortality at either 0.25 ppm or 0.5 ppm of aflatoxins. Garlic at the level of (4%) decreased the mortality in aflatoxicated birds (grp 9 vs. 5) from 9.33% to 2.66% (which received 0.5 ppm AF), and from 8.0% to 2.66% when birds received AF at a level of 0.25 ppm (grp 8 vs. 6). Garlic at the level of 2% decreased the mortality from 9.33% to 4.0% when birds received 0.50 ppm AF(grp 9 vs. 7) and also when birds received aflatoxin at 0.25 ppm (grp 8 vs. 4) the mortality decreased from 8.0% to 5.33%.

Biochemical results

A. Serum proteins and albumin

(1) Testing at the age of 28 days of age (Table 4):

The decrease in the amount of total protein (TP) was directly proportional to the concentration of aflatoxin (grp 1,8,9). While the increase of TP and cholesterol were directly proportional to the addition of garlic.(grp 1,2,3). The effect of aflatoxin at 0.5 ppm in feed decreased significantly ($p<0.001$) TP (grp 5,7,9). The addition of garlic even at low level (2%) was effective in restoring the decrease of the TP as seen in grp 9 as compared with grp 7.

(2) Testing at 49 days of age (end of experiment):

As with the results seen by testing at the age of 28 days the same correlation between AF level and the tested parameters was seen.

Total serum protein and albumin were significantly inversily proportional to the increase of aflatoxin incorporation.grp 8,9.

In grp 7,9 the effect of garlic (4%) was evidenced by a significant increase in the total serum protein and albumin

B. Enzymes

(1) Testing at the age of 28 days of age (Table 4) :

In the non toxicated groups which received garlic (grp 2,3), non significant reductionin in the concentration of ALT enzyme was reported in comparison with the non toxicated and non garlic treated (grp 1).

Also with AST, garlic addition at both rates caused a reduction although non significant in the level of this enzyme in non toxicated groups.

Regarding AST, the data indicated that AF had a deteriorating effect (grp 1 vs. grp 8,9). Meanwhile addition of garlic either at 2% or 4% to aflatoxicated feed had no effect and there was no significant difference in groups (4,5,7). There was only a significant effect for addition of 4% garlic to group received 0.25 ppm AF in the feed (grp 7).

The significant ($p<0.001$) benefit effect of high rate of addition of garlic (4%) on preventing the damage of the liver tissue can be seen by comparing grp 7,9 which received only 0.25 ppm AF.

(2) Testing at 49 days of age (end of experiment):

The picture of enzymatic changes were the same as the age 28 days but more severe in the older age.

C. Cholesterol

(1) Testing at the age of 28 days of age (Table 4):

Treatment with garlic alone increased cholesterol concentration in blood.

Cholesterol was increased in the intoxicated groups with the same level.

Garlic had no significant effects in reducing the cholesterol level.

(2) Testing at 49 days of age (end of experiment):

The effect of AF on cholesterol concentration after 5 weeks of treatment was contrary to the effect after 2 weeks of treatment. Af decreased the concentration of cholesterol in serum which was dose dependent.

Addition of garlic caused but non significantly the concentration of cholesterol.

D. Triglycerides

(1) Testing at the age of 28 days of age (Table 4):

Triglycerides was significantly increased in the intoxicated groups (grp 8&9). Addition of garlic at 4% significantly increased AF at 0.25 ppm. Also addition of garlic at 2% had preservative effect appeared through decreasing the triglycerides in blood.

(2) Testing at 49 days of age (end of experiment):

AF significantly increased the concentration of triglycerides in the serum. Although in the group receiving 0.5 ppm AF the triglycerides were much lower than the group received 0.25 ppm AF.

Hematocrit and hemoglobin concentrations at 49 days of age (Table 5):

Both levels of aflatoxin (grp 8,9) decreased the hematocrit and the hemoglobin concentration significantly in comparison with control grp 1.

The low level of garlic (2%) increased the level of hemoglobin and hematocrit percentage significantly at the both the low AF (0.25 ppm) (grp 4,8) and the high AF (0.50 ppm) (grp 7,9) but still under the normal level of control grp 1. Also the high level of garlic (4%) where both the concentrations of hemoglobin and percentage of hematocrit at both the low and the high level of aflatoxin (grp5&9)

Pathological results

Upon necropsy of the intoxicated birds, the liver was the target organ. It appeared greatly enlarged in birds intoxicated with 0.5ppm AF (grp 9) or slightly enlarged in birds that received 0.25ppm AF (grp 8), those which were given 0.5ppm AF besides 2% garlic (grp 7) or birds which were adminstrated 0.5ppm AF + 4% garlic (grp 5). In addition, the liver of these intoxicated bird groups appeared yellow in color, friable in texture with greasy cut-surface. On the other hand, birds of the remaining groups showed normal liver.

Microscopically, the livers of grp 9 birds suffered from diffuse advanced or severe fatty degeneration of hepatocytes giving the characteristic signet-ring appearance (Fig. 1). The degenerated hepatocytes became swollen, rounded and without cytoplasm which was replaced by fat that dissolved in xylene during processing of the specimen. Moreover, the nucleus was pushed to the periphery of the cell and became flattened. The same picture was noticed in the liver of grp 8 birds but in a localized distribution wherein the periportal hepatocytes were severly degenerated. However, both the centrilobular and midzonal hepatocytes were normal (Fig. 2). Upon adminstration of garlic, the intoxicated groups (7 or 5) exhibited less dramatic picture in the liver which showed only multifocal mild fatty change of some hepatocytes. The affected cells did not change in

shape or in size but their cytoplasm contained singly or multiple small vacules indicating early fatty change (Fig. 3). The intoxicated birds with 0.25ppm AF and treated with 2% garlic (grp 4) or 4% garlic (grp 6) showed normal liver (Fig. 4). Furthermore, the microscopic examination of the liver of the remaining bird groups revealed that it was within the normal histologic limits as the control grp (Fig. 5).

Effect of garlic treatment during aflatoxicosis on the immune response against Newcastle vaccination (Table 6)

Garlic at 2% and 4% gave the highest HI antibody titer response (grp 2&3). Aflatoxin itself did not affect the antibody titers in the other toxicated or toxicated and treated groups. There was no significant reduction in the immune response in the group received 0.25 ppm or 0.50 ppm aflatoxin.

Effect on relative organ weights

Regarding the effect of garlic on the relative organ weight (Table 7) it is clear that after 2 weeks of treatment, aflatoxin increased the relative weight of liver to the body weight in groups received either aflatoxin alone or aflatoxin and garlic. This was not the case with either intestine or with Bursa of Fabricius as the difference among groups was not significant.

After 3 and 5 weeks of treatment there was no significant difference among the relative weights of liver, spleen and bursa of Fabricius to the body weights.

Discusion

Aflatoxin is expected to be present in feed under wide range of conditions at almost allover the year because the genus *Aspergillus* is ubiquitous in nature, in the soil, in the grains, and in the air. It produces marked economical losses in poultry industry. Trace amounts of aflatoxin can deteriorate the biochemical values of the body (Schell et al., 1993)

Because it inhibits DNA and RNA transcription, inhibits DNA-dependent RNA polymerase and causes impairment of nuclear DNA template function and thus preventing protein synthesis. (Yu, 1977 and Shaaban et al., 1991). These effects will lead to decrease in the body weight of birds and decrease effeciency of feed conversion.

Through this mechanism AF also causes decrease in the values of haemoglobin and haematocrite.

The liver is the site of synthesis of serum protein (albumin and globulins), triglycerides and cholesterol so the effect of aflatoxicosis as hepatotoxin can be expected (Beers et al., 1990). The histopathological changes of examined organs in all aflatoxin-trated groups appeared to be a dose related. Hepatic cell fatty degeneration, cytoplasmic

vacoulization were present in varying degrees.

The deteriorative effects of aflatoxins on the different liver function and blood parameters studied were significantly produced in both groups (grp 8&9) received only intoxicated feed.

The level of albumin decreased. This is in accordance with the results of Abd El-Hamid et.al., (1992) on the laying quails. Also the total serum protein and globulin concentrations were all decreased.

Triglycerides was significantly increased in the intoxicated groups (grp 8&9) after both 2 and 5 weeks of treatment. This is due to disturbance in the fat metabolism and inability of the liver to assimilat these products. AF caused deformed lipogenesis in liver. (Harvey et al., 1993).

Although the increase was much higher in 0.25 ppm AF toxicated group than that intoxicated with 0.5 ppm AF. The same observation was seen at the end of the experiment. This could be explained by the fact that both doses of AF increased the lipogenesis in liver but the higher dose of AF caused cirrhosis later on which destructed completely the hepatic tissue.

toxicated feed.

Aflatoxin increases the level of AST and ALT enzymes in bird sera. Our results are in disagreement with previous reports who mentioned that the activity of different anabolic and catabolic enzymes as (LDH), ALT, sorbitol dehydrogenase and glutamic dehydrogenase were decreased in cases of aflatoxicosis (Tung et al., 1972, Doerr et al., 1983; Huff et al., 1986, Dafalla et al., 1987, Abd El-Hamid et al., 1992, Fernandez et al., 1994). The higher levels of enzymes AST (GPT) and ALT (GOT) in the serum may indicate liver tissue damage and subsequently leads to the leakage of such enzymes to the blood.

The presence of ALT means acute inflammation, while the AST enzyme increases in cases of chronic inflammation (cirrhosis) of the liver. The data affirm this fact as the level of AST was increasing towards the end of the experiment. After 2 weeks of treatment, it was clear from grp 5&9 that effect of the high level of garlic (4%) with 0.50 ppm AF was significant in reducing the damage of the liver tissue as indicated by the lowering of the level of this enzyme. In comparing groups 6&8, the significant decrease in ALT level due to 0.25 ppm AF was prominent

But the differences in the values of total serum proteins, AST, triglycerides, and cholesterol at the first reading after 2 weeks of treatment (at the age of 28 days) and the second reading after 5 weeks of treatment (at the age of 49 days) could be attributed to the increase in the body and liver weights due to maturation.

Regarding haemoglobin and haematocrite values, an increase in their values was parallel to the garlic inclusion in the feed. This indicates the positive effect of garlic on both

blood parameters which are the reflection of healthy conditions of liver and spleen as well as other tissues like bone marrow where red blood cells are synthsized.

Regarding the effect of garlic on the relative organ weight it was clear that after 2 weeks of treatment aflatoxin increased the relative weight of liver to the body weight in groups received either aflatoxin alone or aflatoxin and garlic. These results indicate that the change in liver weight due to aflatoxicosis was more pronounced than other organs and assure that liver is the most susceptible organ for aflatoxicosis.

This was not the case with either intestine or with Bursa of Fabricius as the difference among groups was not significant. This also goes in parallel with the results of the HI titers to NDV where aflatoxins has no significant adverse effect on the antibodies produced from the bursa of Fabricius.

After 3 and 5 weeks of treatment there was no significant difference among the relative weights of liver, spleen and bursa of Fabricius to the body weights. This means that organ weights changed in parallel manner to the change of the body weight among different groups according to the different treatments. As there was a significant effects of garlic treatment on the body weight, we can conclude that garlic treatment significantly corrected the adverse effects on organ weights.

On the other hand, garlic was reported to contain allicin (diallyldisulfide-S-oxide) which is an important antibacterial agent and an antithrombotic factor designated ajoene which inhibit the fibrinogen receptors on the blood platelets (Tyler et al., 1988). This antithrombotic action may be the reason of preservative effect of garlic against haemorrahge caused by AF, thus leading to decreased toxicity.

These beneficial effects of garlic led to better metabolism amd better utilization of nutrients.

This was reflected by significant dose related improvement in all parameters studied such as serum protein, albumin, globulin, haematocrite, haemoglobin, different enzymes, individual organ weight, mortality, body weight and feed conversion (grp 2&3).

Addition of garlic to the feed of intoxicated birds significantly improved the performance and alleviated the adverse effects of AF. Garlic decreased but not significantly these two enzymes ALT and AST when used in non toxicated feed.

Also garlic corrected the disturbed enzymatic activities of the liver. Garlic also diminished significantly the mortality due to aflatoxins. Although garlic showed non significant higher antibody titers in both groups received no aflatoxins, but the aflatoxin itself did not affect the antibody titers in the toxicated groups.

We can conclude that the inclusion of garlic in the feed of poultry at the rate of 2%-4% improved the feed conversion, body weight, and

biochemical measurements in the blood of Japanese quails.

Acknowledgement

Thanks go to Prof. H.S. Abd El-Hamid, Dept. of Avian and Aquatic Animal Med., Fac. Vet. Med., Alexandria Univ. for providing the standard toxigenic strain of A.flavus and for helping in estimation of the level of aflatoxins in experimental feed.and for Dr. M.M. El-Habbak professor of poultry physiology, Dept. of poultry production, Kafr El-Sheikh, Fac. Agriculture, Tanta Univ. for his continuous help and offering all facilities needed for completion of this study.

Also the authors are appreciated for the help of Dr. Yousef, S. lecturer of Pathology,.Fac. Vet. Med. Alexandria Univ.in reading the histopathological section.

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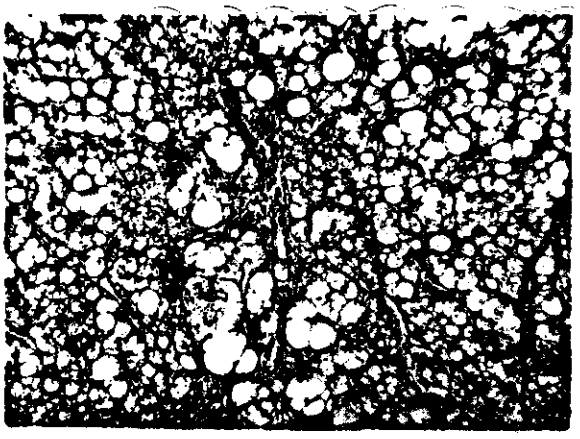

Fig. (1): liver of a group 9 bird: Diffuse advanced fatty change of the hepatocytes characterized by signet-ring appearance. H,E. (x250).

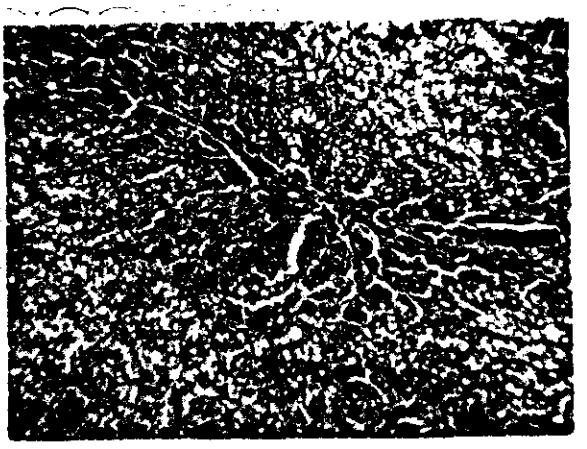

Fig. (2): liver ofa group 8 bird: Focal advanced fatty change of the periportal hepatocytes (arrows) and the centrilobular and midzonal hepatocytes were not affected (A). H,E. (x160).

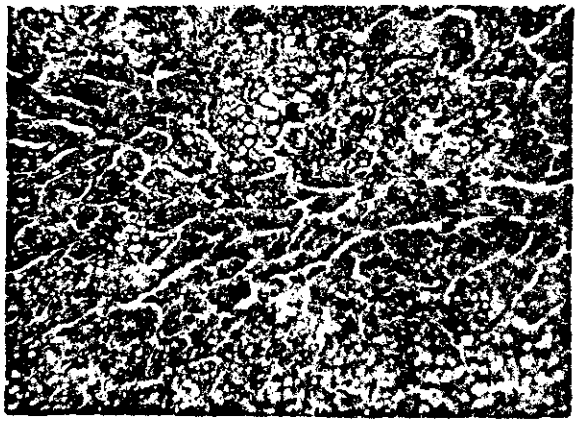

Fig. (3): liver of a group 5 bird: Early fatty change of some hepatocytes (arrows) characterized by cytoplasmic vacuolation. H,E. (x160).

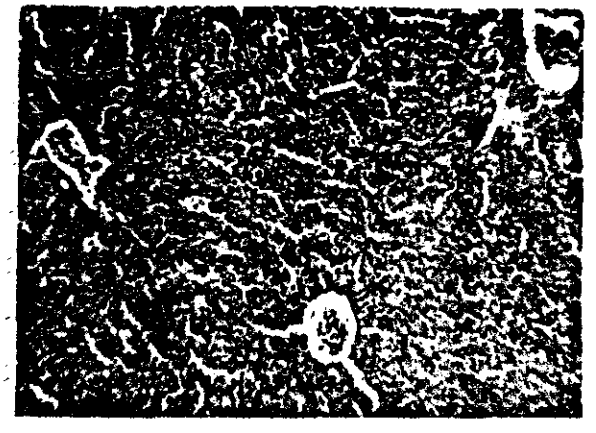

Fig. (4): liver of a group 4 bird: Norma hepatocytes. H,E. (x160)

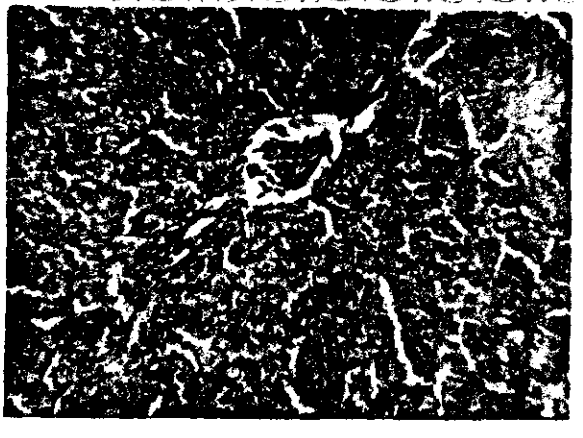

Fig. (5): liver of a group 3 bird: Normal hepatocytes. H,E. (x160)

Table (1) **The single and combined effects of garlic (Allivum Sativum) and aflatoxin on body weight of growing Japanese quail .**

Grp No.	Treatments		Body weight (g) (X ± S . E) .					
	Garlic (%)	AF ppm)	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week
1	0	0	45.34 ± 1.18	65.89± 1.72	95.97 ±2.61	120.88 ±2.78	137.06 ±3.06	159.78± 3.99 b
2	2%	0	43.11±0.96	66.& ± 1.52	93.88± 2.54	120.52 ± 2.87	151.52 ± 3.51	168.31 ± 3.43 b
3	4%	0	40.94 ± 1.10	67.82 ± 2.09	99.20 ± 2.47	124.44 ± 2.98	156.48 ± 2.73	180.22 ±3.26 a
4	2%	(0.25)	45.75 ± 0.87	64.61 ± 1.69	95.72 ± 2.66	120.28± 3.37	145.28 ± 3.66	160.67 ± 3.60 b
5	4%	(0. 50)	44.31 ± 1.12	64.91 ± 1.99	97.39 ± 3.20	115.74 ± 3.51	146.07 ± 3.03	160.72 ± 2.53 b
6	4%	(0.25)	46.26± 1.32	65.66 ± 2.18	99.93 ± 4.05	120.53 ± 2.81	142.90 ± 3.51	159.12 ± 3.86 b
7	2%	(0.50)	41.42 ± 0.93	63.00 ± 1.54	90.62 ± 2.60	112.46 ± 3.03	134.93 ± 3.57	142.69 ± 3.75 c
8	0	(0.25)	43.65 ± 1.12	67.57 ± 2.51	91.26 ± 2.63	114.74 ± 3.22	129.73 ± 3.79	143.48 ± 4.56 c
9	0	(0.50)	42.05 ± 1.09	61.79± 2.18	85.12 ± 2.95	110.69 ± 3.97	126.52 ± 4.75	136.29 ± 5.33 c
Significance level			**	N. S	N. S	*	***	***

Values are means ± Standard error .

a-e Means within a columns with no Common superscripts differ significantly ($p < 0.05$).

N.S : Non significant . * :$P < 0.05$

**: $P < 0.01$ ***: $P < 0.001$

Table (2) Average values of performance parameters of quail in different groups during experimental period (5 weeks)

Grp No.	Treatments		Total body weight gain (g)	Total feed intake (g / bird)	Feed conversion
	Garlic(%)	Aflatoxin (ppm)			
1	0	0	116.35±3.45 bc	699.47±7.88de	5.95±0.08c
2	2	0	126.00±3.07b	696.48±3.16de	5.44±0.03d
3	4	0	140.09±3.39a	682.54±9.19e	4.87±0.06e
4	2	0.25	116.33±3.72bc	720.69±2.20cd	6.19±0.05c
5	4	0.50	177.56±2.64bc	737.71±19.35bc	6.28±0.24c
6	4	0.25	113.77±3.18c	777.01±9.94a	6.83±0.12b
7	2	0.50	101.61±3.39d	790.08±5.77a	7.83±0.12a
8	0	0.25	99.89±3.99d	778.97±14.80a	7.79±0.15a
9	0	0.50	95.12±5.13d	767.22±9.93ab	8.06±0.05a
Significance level			***	***	***

Values are means ± standard error .
a-d Means within a columns with no cammor superscripts differ significantly ($p< 0.05$)
*** $P < 0.001$

Table (3) Elfect of experimental treatments on number of mortality percent of growing Japanese quail

Grp No.	Garlic (%)	AF (ppm)	Number of mortality	Mortality(%)
1	0	0	2	2.66
2	2%	0	2	2.66
3	4%	0	2	2.66
4	2%	0.25	4	5.33
5	4%	0.50	2	2.66
6	4%	0.25	2	2.66
7	2%	0.50	3	4.00
8	0	0.25	6	8.00
9	0	0.50	7	9.33

Table (4) The single and combined effects of garlic and aflatoxin on the plasma concentrations of total pro tein, albumin, globulin, cholesterol, triglycerides, asparatate aminotransferase (AST) and alanine aminotransferase (ALT) of japanese quail

Grp No.	Treatments		Total protein	Albumin	Globulin	Cholesterol	Triglycerides	AST	ALT
	Garlic %	AF (ppm)	g/dl	g/dl	g/dl	mg/dl	mg/dl	u/l	U/l
At 28 day of age									
1	0	0	3.10±0.06 c	1.33 ±0.09 b	1.77 ±0.15 cd	94.00±10.97 ef	74.00 ± 0.00 c	53.00± 13.00 c	6.00± 0.15 ab
2	2%	0	3.53± 0.20 b	1.37 ± 0.09 b	2.17± 0.29 abc	212.5±5.49f	125.50± 10.68 c	46.67 ±10.36 c	4.00 ± 0.00 bc
3	4%	0	4.10 ± 0.00 a	1.50 ± 0.00 ab	2.60 ± 0.00 a	153.00±5.49bc	88.33 ± 4.33 c	49.33 ± 1.45 c	3.33 ± 0.33 c
4	2%	0.25	4.00 ± 0.06 a	1.70 ± 0.12 a	2.30 ± 0.06 ab	173.00 ±4.62 b	92.50 ± 37 b	93.00 ± 4.72ab	4.33 ± 0.88 bc
5	4%	0.50	2.30± 0.06 c	1.07±0.09c	1.25± 0.15abc	173.00±14.75a	198.50±8.37 b	91.66± 9.53ab	2.00± 0.00 a
6	4%	0.25	2.77± 0.29 cd	0.77± 0.09 de	2.00±0.23 bcd	142.33±10.11cd	221.50±0.00b	73.33±12.41bc	6.00±0.00ab
7	2%	0.50	3.00±0.00e	0.77±0.09c	2.25± 0.09e	84.00± 4.62b	125.50± 779a	91.66±9.53ab	7.00±1.73c
8	0	0.25	2.50±0.06dc	0.90±0.00cd	1.60± 0.06 de	115.00±5.49de	422.00±12.70a	92.00± 1.73 ab	4.00 ± 0.00 bc
9	0	0.50	1.90± 0.06 f	0.60 ± 0.00 e	130.0.06 e	115.00±12.99de	140.004.33 a	107.33± 7.22 a	4.00± 0.00 bc
Significance level			***	***	***	***	***	***	***
At 49 day of age									
1	0	0	4.70 ± 0.29 a	1.43 ± 0.10 a	3.27 ± 0.39 a	153.00 ± 8.08ab	164.00 ±0.00b	49.67± 1.45 e	4.67 ± 0.88 b
2	2%	0	3.73± 0.26 b	1.17± 0.22 a	2.57±0.09 bc	105.33± 3.18 c	94.00± 0.00de	71.67± 2.60 d	4.67± 0.88 b
3	4%	0	3.73± 0.09 b	0.47± 0.08 bc	3.27± 0.03 a	136.00± 0.78abc	823.00± 3.46 e	59.67± 4.33de	3.67± 0.33 bc
4	2%	0.25	3.60± 0.00 b	0.03± 0.00 c	3.30± 0.00a	163.67± 14.14a	276.00±20.11 cd	88.00± 6092 c	5.00± 0.57 b
5	4%	0.50	4.03± 0.09 b	0.47± 0.09 b c	3.57± 0.03 a	114.00± 1.73c	235.00± 0.00 a	120.00± 0.00 a	6.00± 1.15 b
6	4%	0.25	2.50± 0.29 c	0.030± 0.00 c	2.20± 0.29 c	119.00± 14.43c	552.00± 1.00 b	97.00± 11.53 bc	2.00 ± 0.00 c
7	2%	0.50	3.43± 0.09 b	0.47± 0.09 bc	2.97± 0.18 ab	128.00± 0.00bc	529.00± 9.53 b	89.00± 0.00 c	4.33± 0.88 bc
8	0	0.25	2.87± 0.24 c	0.77± 0.09 b	2.10± 0.30 c	108.67 ± 4.9 c	376.00±21.13 bc	120.00± 0.00 a	10.00± 1.15 a
9	0	0.50	2.40± 0.00 c	0.30± 0.00 c	2.10± 0.00 c	72.00± 00 d	76.00± 27.13a	110.00± 5.78 ab	9.00± 0.58 a
Significance level			***	***	***	***	***	***	***

Values are means ± stand error

*** : $p < 0.001$

Table (5) The single and combined effects of garlic and aflatoxin on the hemoglobin concentration (Hb) and haematocrite (Ht) of growing Japanese quail at 49 days of age

Grp No.	Garlic %	Aflatoxin (ppm)	Hemoglobin (Hb) 9% dl	Haematocrite (Ht) %
1	0	0	12.90 ± 0.00 a	36.00 ± 0.58 a
2	2%	0	10.10 ± 0.12 e	29.67 ± 0.33 d
3	4%	0	12.90 ± 0.00 a	37. 0.00 ± a
4	2%	0.25	11.20 ± 0.25 c	32.00 ± 0.58 c
5	4%	0.50	11.73 ± 0.20 b	34.00 ± 0.57 a
6	4%	0.25	12.50 ± 0.23 a	36.00 ± 0.58 a
7	2%	0.50	10.63 ± 0.20 d	31.00 ± 0.57 cd
8	0	0.25	7.20 ± 12 g	22.00 ± 0.57 e
9	0	0.50	8.40 ± 0.23 f	22.00 ± 1.15 e
Significance level		***	***	***

Values are means ± standard error *** : P < 0.00 I

Table (6) The single and combined effects of garlic and aflatoxin on the geometric mean aantibody HI titers (log 2) to vaccination with ND (NewCastle disease virus)

Grp No.	Treatments		ND titers at ages of			
	Garlic %	AF (ppm)	14 ds. (before vaccination)	28 ds.	35 ds.	49 ds.
1	0	0	0.2	2.4	1.6	0.4
2	2%	0	0.2	3.6	2.2	1.0
3	4%	0	0.2	3.6	2.2	1.6
4	2 %	(0.25)	0.4	2.0	1.6	0.8
5	4%	(0.50)	0.2	2.0	1.6	0.8
6	4%	(0.25)	0.0	2.0	1.4	0.6
7	2%	(0.50)	0.2,	1.6	1.2	0.6
8	0	(0.25)	0.2	2.2	1.0	0.8
9	0	(0.50)	0.2	2.0	1.2	0.6

Table (7) Effect of treatments on the weight of immune organs relative to live body weight of growing Japanese quail

Grp	Treatments		Organs weight (%) At 28 days of age			
	Garlic %	AF (ppm)	Small intestine	Liver	Spleen	Bursa of fabricius
1	0	0	7.26 ±0.54	2.25 ± 0.13 c	0.054 ± 0.006 bc	0.117 ± 0.015
2	2%	0	7.39 ± 0.65	2.76 ± 0.31abc	0.065 ± 0.008 abc	0.114 ± 0.015
3	4%	0	7.33 ± 0.32	2.43 ± 0.26 b c	0.050 ± 0.007 c	0.150 ± 0.023
4	2 %	(0.25)	7.32. ± 0.53	2.76 ± 0.13 abc	0.077 ± 0.012.abc	0.094 ± 0.010
5	4%	(0.50)	6.833 ± 0.55	3.72 ± 0.22 a	0.098 ± 0.017 a	0.097 ± 0.013
6	4%	(0.25)	6.96 ± 0.56	3.52 ± 0.34 a	0.082 ±0.009 abc	0.110 ± 0.024
7	2%	(0.50)	6.83 ± 0.55	3.33 ± 0.44ab	0.093 ±0.617ab	0.108 ± 0.008
8	0	(0.25)	6.61 ± 0.49	3.36 ± 0.48 ab	0.097 ± 0.019 a	0.089 ± 0.007
9	0	(0.50)	6.41 ± 0.36	3.67 ±0.14 a	0.0079±0.004 abc	0.097 ± 0.012
Significance level			N . S	**	*	N . S

Valnes are means ± standard error
N.S.: non significant
** P < 0.001

Table (7) Cont.

Grp No.	Treatment		Organ weight At 35 day of age			
	Garlic %	AF (ppm)	Small intestine	Liver	Spleen	Bursa of fabricius
1	0	0	5.76 ± 0.30	2.73 ± 0.26	0.027 ± 0.003	0.070 ± 0.013
2	2%	0	5.49 ± 036	2.75 ± 0.29	0.070 ± 0.004	0.092 ± 0.009
3	4%	0	5.14 ± 0.29	2.45 ± 0.16	0.043 ± 0.010	0.089 ± 0.014
4	2%	(0.25)	5.27 ± 0.29	3.02 ± 0.19	0.068 ± 0.0013	0.073 ± 0.018
5	4%	(0.50)	4.88 ± 0.43	2.31 ± 0.37	0.069 ± 0.007	0.081 ± 0.008
6	4%	(025)	5.30 ± 0.43	3.36 ± 0.31	0.066 ± 0.016	0.0088 ± 0.009
7	2%	(0.50)	4.96 ± 0.26	3.08 ± 0.22	0.085 ± 0.011	0.062 ± 0.022
8	0	(0.25)	4.97 ± 0.24	3.33 ± 0.29	0.070 ± 0.018	0.063 ± 0.013
9	0	(0.50)	5.37 ± 0.82	3.34 ± 0.43	0.056 ± 0.017	0.050 ± 0.013
Significance level		-	N.S.			
Grp No.	Garlic %	AF (ppm)	Organ weight At 49 day of age			
			Small intestine	Liver	Spleen	Bursa
1	0	0	6.20 ± 0.08	3.34 ± 0.39	0.048 ± 0.006	0.075 ± 0.015
2	2%	0	7.07 ± 0.25	3.09 ± 0.21	0.067 ± 0.009	0.077 ± 0.014
3	4%	0	6.53 ± 0.39	3.39 ± 0.19	0.070 ± 0.003	0.086 ±0.019
4	2%	(0.25)	5.33 ± 0.29	3.04 ± 0.32	0.098 ± 0.010	0.081 ± 0.011
5	4%	(0.50)	5.31 ± 0.25	3.57 ± 0.09	0.082 ± 0.025	0.058 ± 0.029
6	4%	(0.25)	5.50 ± 0.68	2.86 ± 0.51	0.064 ± 0.016	0.071 ± 0.004
7	2%	(0.50)	5.17 ± 0.60	3.06 ± 0.59	0.063 ± 0.021	0.043 ± 0.008
8	0	(0.25)	5.80 ± 0.33	3.91 ± 0.39	0.094 ± 0.018	0.067 ± 0.006
9	0	(0.50)	5.74 ± 0.77	3.38 ± 0.56	0.087 ± 0.023	0.067 ± 0.023
Significance Level			N.S	N.S	N.S	N.S

Valnes are means ± standard error
N.S.: non significant